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Synthesis of brominated isobenzofuranones as new potent inhibitors of quorum sensing

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서울대학교 대학원
화학부 유기화학 전공
李 林 子

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Abstract

Synthesis of brominated isobenzofuranones as new potent inhibitors of quorum sensing

Li Linzi

Department of Chemistry

Seoul National University

We designed and synthesized a series of brominated isobenzofuranone derivatives. These Compounds have significant biofilm inhibitory activities and are good candidates for quorum sensing inhibitors (QSIs). All of them can be synthesized simply within two steps. The optimization of the first step, gem-dibromoolefination of phthalic anhydride, was done by replacing PPh_3 with $P(O\textit{i}\text{-}Pr)_3$ and adding Zn dust, which significantly increased the reaction yields. After Stille coupling of an aryltin compound with the dibrominated furanone (A), a Z form isomer was obtained as a major product. The formation of the Z isomer was confirmed by ^1H NMR spectroscopy and also single-crystal X-ray diffraction. According to the chemistry developed, a series of aryl-substituted bromofuranones were prepared and tested against quorum sensing inhibition. Out of these compounds tested, phenyl-substituted compound proved to be the most active one.

Keywords: Quorum sensing, Periodontitis, Bacterial biofilm, brominated furanones

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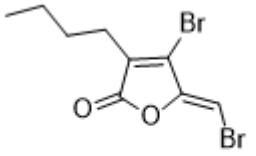
Synthesis of brominated isobenzofuranones as new potent inhibitors of quorum sensing

Introduction

With emerging antibacterial multidrug resistance [1], traditional treatment of diseases is stagnating. Development of new therapeutic methods is being actively pursued all over the world. Recently a great deal of interest has been concentrated to the cell-to-cell communication among bacteria. This communication system, termed as quorum sensing (QS), depends on the density of bacteria population and works by detecting autoinducers. It controls many physiological activities including bioluminescence [2], symbiosis, virulence, motility, sporulation and biofilm formation [3]. Therefore inhibition of QS has become one of the most promising therapeutic approaches for disease control [4].

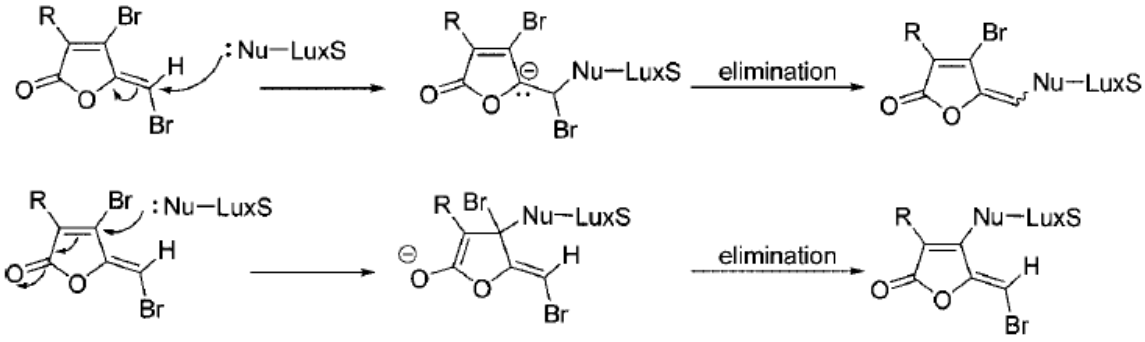
QS inhibitors (QSIs) reported so far can be mainly divided into three classes: proteins, peptides and nonpeptide small molecules. They can inhibit QS by acting as structural analogues of autoinducer receptors. Gram-negative bacteria use acylated homoserine lactones while Gram-positive bacteria use oligo-peptides as autoinducers to communicate within species. However, recent advances have shown that autoinducer-2 (AI-2) is an excellent QS molecule widely produced by various bacteria and can be used in intra-species as well as inter-species communication [5–7]. For example, AI-2 is the primary QS molecule produced by more than 700 species of oral bacteria as suggested by Kolenbrander [8]. He proposed that pathogens produce and receive much higher level of AI-2 than commensal bacteria, which enhances biofilm formation thus causing periodontitis.

Synthesis of AI-2 molecules is related to the activity of enzyme LuxS. Zang et al. have reported that some natural brominated furanones can inhibit LuxS. The proposed mechanism is shown below (scheme 1) [9,10].



natural brominated furanone

Scheme 1: **mechanism proposed by Zang et al.** [9,10]



As our previous study has reported, AI-2 produced by Fusobacterium nucleatum, the main periodontopathogen, was reduced by QSIs [6]. It represents a new effective therapeutic strategy for periodontitis using QSI without disturbing other numerous oral commensals. Recently we also reported a new class of AI-2 quorum-sensing inhibitors against bacterial biofilm formation [11]. It showed that the bicyclic structure is essential for good inhibitory activities. After succeeding in the synthesis of bicyclic dibrominated furanones, we have continued our investigation on new potent and safer candidates. We synthesized a series of monobrominated furanone derivatives that retain the bicyclic structure and present the QS inhibition.

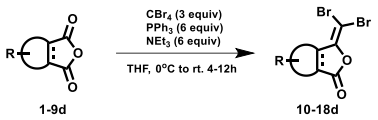
I. Bicyclic dibrominated isobenzofuranones

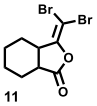
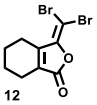
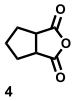
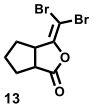
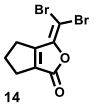
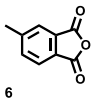
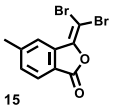
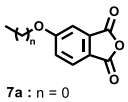
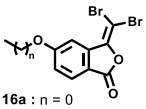
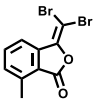
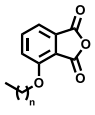
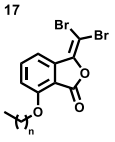
1. Chemistry

We first designed and synthesized bicyclic dibrominated furanone derivatives with low toxicity but improved biofilm inhibitory activities. We synthesized two kinds of bicyclic brominated furanone derivatives by modification of the ring structure and side chain on the benzene ring. The ring structure has been reported to be important for good inhibition of biofilm formation [12]. Thus, compounds with different ring structures (**QS-D:1–5**) were designed for evaluation of the ring structure effect on the biofilm inhibitory activity. It has also been reported that monocyclic furanones with chain lengths of two to six atoms have good inhibitory activities [13,14]. Therefore, in order to investigate the effect of side chains, we designed another set of compounds with different chains on benzene ring

According to the gem-dibromoolefination procedure [15], brominated compounds with various ring structures (**QS-D:10–14**) shown in Table 1 were synthesized from the corresponding commercially available compounds. The yields ranged from low to moderate (24–65% yields). However, the olefination products of cyclobutane-1,2-dicarboxylic anhydrides decomposed rapidly at room temperature presumably due to the unstable nature of the anhydrides based on the ring strains of the hydrocarbon ring [16].

Table 1. **Synthesis of bicyclic dibrominated furanone derivatives(QS-D).**



Entry	Substrate	Product	Yield (%)[a]
1	 **1**	 **10**	30%
2	 **2**	 **11**	40%
3	 **3**	 **12**	65%
4	 **4**	 **13**	24%
5	 **5**	 **14**	27%
6	 **6**	 **15**	24%
7	 **7a** : n = 0 **7b** : n = 1 **7c** : n = 2 **7d** : n = 3 **7e** : n = 4	 **16a** : n = 0 **16b** : n = 1 **16c** : n = 2 **16d** : n = 3 **16e** : n = 4	**16a** : 22% **16b** : 20% **16c** : 19% **16d** : 21% **16e** : 19%
8	 **8**	 **17**	26%
9	 **9a** : n = 1 **9b** : n = 2 **9c** : n = 3 **9d** : n = 4	 **18a** : n = 1 **18b** : n = 2 **18c** : n = 3 **18d** : n = 4	**18a** : 19% **18b** : 18% **18c** : 18% **18d** : 16%

[a] yields of isolated products

The substrates containing alkoxy groups on the benzene ring **QS-D: 7a-7d** and **QS-D: 9a-9d** were prepared from the corresponding phenol derivatives at moderate to good yields over four steps (45%-77%). Dibrominated compounds **QS-D: 15-18d** with side chains on the benzene ring were obtained with 70-83% regioselectivity. As a similar study that examined the regioselectivity of Wittig reactions for phthalic anhydrides [17], this result showed that the electronic effects of the substituents appear to have a big influence on the regioselectivity. Compounds **QS-D: 15**–**18d** were obtained in overall 16% to 22% yields. Small amounts of minor isomers were produced from the anhydrides **QS-D: 6**–**9d**. However, we focused on the major products because they have much better inhibitory activity. All compounds were purified through column chromatography. The structures were all confirmed by ^{1}H, ^{13}C NMR, heteronuclear multiple bond correlation (HMBC), and mass spectrometry (MS).

2. Biological assays

In order to evaluate the inhibitory activities of these dibrominated furanones we synthesized, they were first screened for their inhibitory effects on the biofilm formation of F. *nucleatum* at 0.2 and 2 μM concentrations comparing with reference furanone compound R1. The Compound with 5-membered ring structure (**QS-D: 13**) and the one with a short methyl chain on the benzene ring (**QS-D: 15** and **17**) showed high inhibitory effects on the biofilm formation than the others. Then we chose compounds **QS-D: 13**, **15** and **17** for further evaluation in the following biological assays.

2.1 Inhibitory effect on the biofilm formation of major periodontopathogens

We tested the inhibitory effect of the three compounds (**QS-D: 13**, **15** and **17**) on

biofilm formation by observation using a confocal laser-scanning microscope. Three different periodontopathogens, *F. nucleatum*, *P. gingivalis*, and *T. forsythia* were grown on a cover slip.

As shown below, compounds **QS-D: 13**, **15**, and **17** at 2 μM significantly reduced the biofilm formation of periodontopathogens induced by *F. nucleatum*, *P. gingivalis*, and *T. forsythia*. The average depth of the biofilm formed on glass cover slips was analyzed using the Carl Zeiss LSM 700 program. Their inhibitory activities were all higher than Compound R1, which was reported as a good QSI, at all concentrations tested (0.002 - 2 μM). The inhibitory activities of compounds **QS-D: 13** and **17** did not follow a dose-dependent tendency. The structure of the AI-2 receptor may be different among bacterial species [10,18]. The furanone compounds may act as partial agonists or antagonists depending on the concentration in accordance with the different structures of AI-2 receptors [19–21]. It is notable that the inhibitory activities of **QS-D: 13**, **15**, and **17** against F. nucleatum biofilm formation are superior to those of the reference furanone compound even at very low concentration (2 nM). The inhibition of biofilm formation can obstruct the formation of a pathogenic biofilm, and thus prevent periodontitis [22–25].

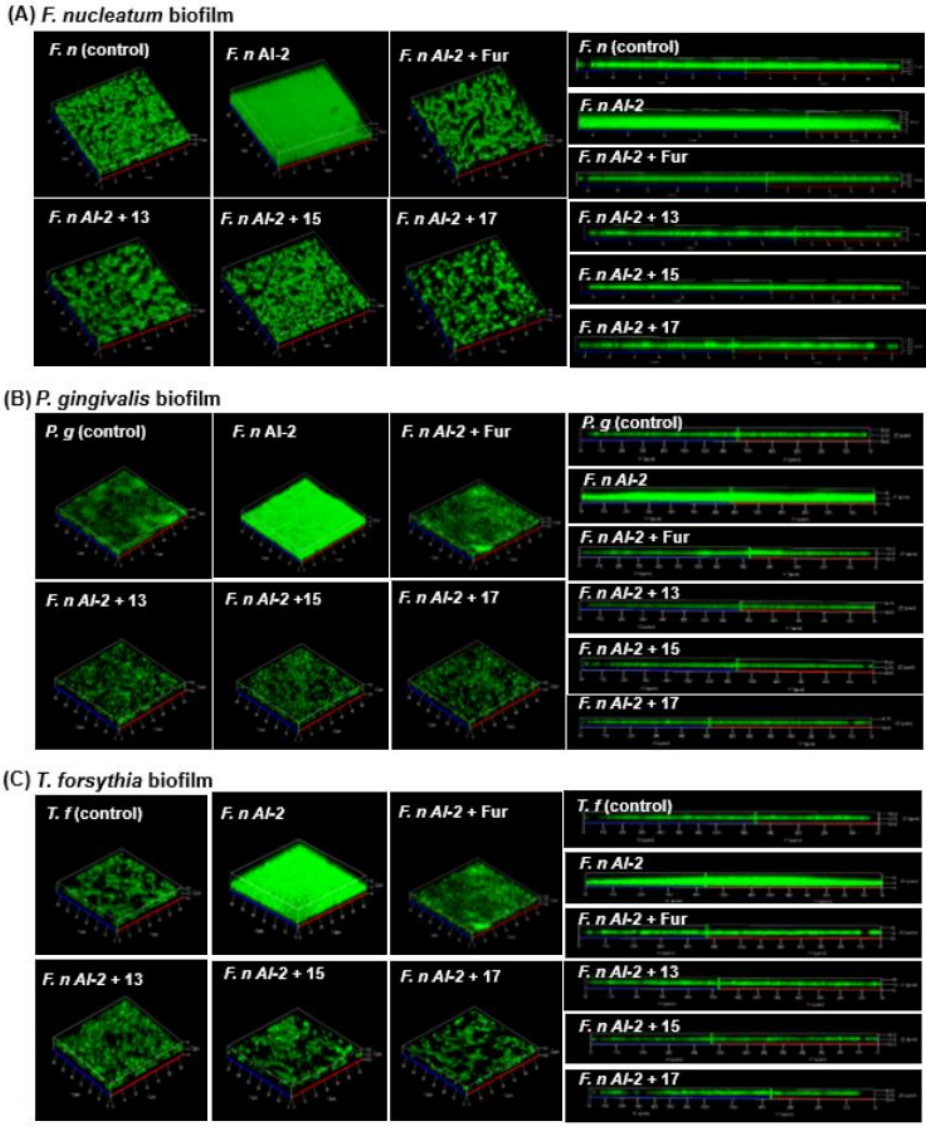


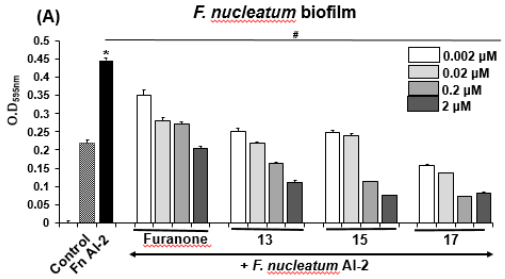
Fig. 1. Biofilm images showing the inhibitory effects of furanone compounds on the biofilm formation of *F. nucleatum, P. gingivalis,* and *T. forsythia.* (A) *F. nucleatum* (2×10^7 bacteria/mL), (B) *P. gingivalis* (2×10^8 bacteria/mL), and (C) *T. forsythia* (2×10^8 bacteria/mL) were cultured with semi-purified *F. nucleatum* AI-2 in the absence or presence of the reference furanone compound (Fur) or new furanone compounds (**QS-D: 13**, **15**, and **17**) at 2 μM under an anaerobic condition at 37°C. After 48 h incubation, each biofilm formed on glass cover slips was stained with the live/dead-BacLight bacterial viability kit followed by observation using a confocal laser-

scanning microscope at 1000× magnification. The control group was the biofilm of each bacterium cultured without *F. nucleatum* AI-2 and inhibitors.

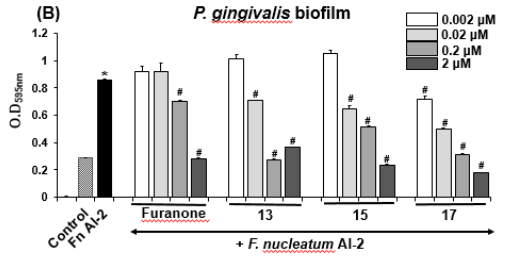
Table 2. Biomass and depth of periodontopathogen biofilms.

Treatment \ Bacteria	*F. nucleatum*		*P. gingivalis*		*T. forsythia*	
	Biomass ($\mu m^3/\mu m^2$)	Average Depth (μm)	Biomass ($\mu m^3/\mu m^2$)	Average Depth (μm)	Biomass ($\mu m^3/\mu m^2$)	Average Depth (μm)
Control	0.43	2.11	0.37	2.06	0.52	2.21
Fn AI-2	2.99*	5.37*	3.73*	4.87*	4.05*	5.29*
Fn AI-2/ Furanone	0.42#	2.02#	0.30#	2.05#	0.77#	2.18#
Fn AI-2/ 13	0.39#	1.82#	0.31#	2.05#	0.93#	2.27#
Fn AI-2/ 15	0.38#	1.87#	0.29#	2.05#	0.59#	1.94#
Fn AI-2/ 17	0.32#	1.72#	0.27#	1.97#	0.88#	2.41#

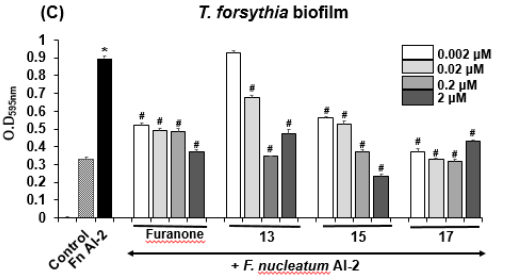
*p < 0.05 compared with the untreated group (Control), and #p < 0.05 compared with the *F. nucleatum* AI-2-treated value in the absence of furanone compounds.



	Biofilm formation (%)			
	0.002 μM	0.02 μM	0.2 μM	2 μM
Fn AI-2	100	100.00	100	100
Furanone	82.56	69.51	68.05	55.61
13	64.02	58.29	47.93	38.78
15	63.90	62.07	39.02	32.07
17	47.07	43.17	31.34	33.17



	Biofilm formation (%)			
	0.002 μM	0.02 μM	0.2 μM	2 μM
Fn AI-2	100	100.00	100	100
Furanone	106.31	106.10	83.09	39.22
13	116.08	84.13	38.46	48.37
15	119.89	77.89	63.83	34.72
17	84.75	61.95	42.97	29.31



	Biofilm formation (%)			
	0.002 μM	0.02 μM	0.2 μM	2 μM
Fn AI-2	100	100.00	100	100
Furanone	62.45	59.71	58.90	47.39
13	103.41	78.18	44.85	57.76
15	66.40	63.05	47.52	33.47
17	47.66	43.04	41.97	53.48

Fig.2. Inhibitory effects of furanone compounds on the biofilm formation of

periodontopathogens at various concentrations. (A) *F. nucleatum* (2×10^7 bacteria/mL), (B) *P. gingivalis* (2×10^8 bacteria/mL), and (C) *T. forsythia* (2×10^8 bacteria/mL) were cultured with 10% semi-purified *F. nucleatum* AI-2 in the presence of the reference furanone compound (Fur) or new furanone compounds (**QS-D: 13**, **15**, and **17**) at various concentrations for 48 h. Biofilm formation was assessed by crystal violet staining. *$p < 0.05$ compared with the non-treated value, and #$p < 0.05$ compared to the *F. nucleatum* AI-2-treated value in the absence of furanone compounds. The experiments were performed three times in triplicate and representative data are shown.

2.2 Inhibitory effect on AI-2 activity

AI-2 activities were evaluated by measuring the AI-2 mediated bioluminescence of the bacteria using Vibrio harveyi BB170, an AI-2 reporter strain. An additional bacterial growth test showed us that the reference furanone compound significantly inhibited the growth of V. harveyi BB170 (Fig. 1B) by reducing bioluminescence. The new compounds **QS-D: 13**, **15**, and **17**, which have high inhibitory activities, were screened for their inhibitory effect on the AI-2 activity comparing with the reference furanone compound. As shown in Fig. 3, They significantly reduced the bioluminescence of V. harveyi BB170 at 2 μM, although their inhibitory activities were lower than that of the reference furanone compound (Fig. 3A). And at the same time, the newly synthesized compounds did not affect the planktonic growth of V. harveyi BB170.

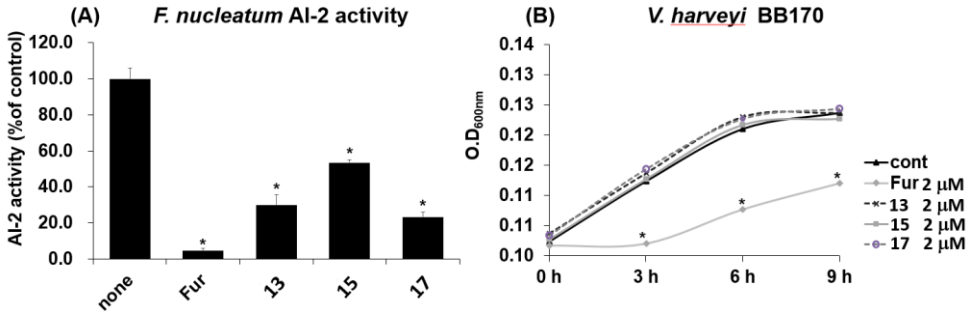


Fig. 3. Effect of furanone compounds on AI-2 activity and *V. harveyi* growth. (A) AI-2 activity was assessed by measuring bioluminescence using the AI-2 reporter strain *V. harveyi* BB170. *V.*

harveyi BB170 (1×10^6 bacteria/mL) was incubated for 6 h with 10% semi-purified *F. nucleatum* AI-2 in the absence or presence of furanone compounds (**QS-D: 13**, **15**, and **17**) and the reference furanone compound (Fur) at a concentration of 2 μM. The bioluminescence of *V. harveyi* BB170 was measured using a luminometer and the value was converted to a percentage of the control value. **p* < 0.05 compared to the value of the *F. nucleatum* AI-2-treated group. (B) *V. harveyi* BB170 was grown aerobically for 9 h in the absence of *F. nucleatum* AI-2 at 30°C in the presence of the reference furanone compound (Fur) or new furanone compounds (**QS-D: 13**, **15**, and **17**) at a concentration of 2 μM. Planktonic bacterial growth was monitored by measuring absorbance at 600 nm using a spectrophotometer. **p* < 0.05 compared to the untreated group (cont). The experiments were performed three times in triplicate and representative data are shown.

2.3 Effect on planktonic bacterial growth

QSIs selectively control virulence expression without disturbing bacterial growth. To confirm the new furanone compounds do not inhibit bacterial growth, we assessed their effects on planktonic bacterial growth. As shown in Fig. 4, the compounds did not inhibit the growth of *F. nucleatum*, *P. gingivalis*, and *T. forsythia* even at the highest concentration (2 μM) in the biofilm formation assay.

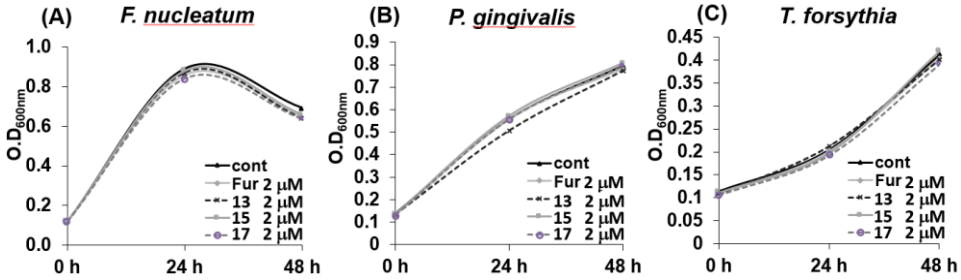


Fig. 4. Effects of furanone compounds on bacterial growth. (A) *F. nucleatum*, (B) *P. gingivalis*, and (C) *T. forsythia* were grown for 48 h under anaerobic conditions at 37°C in the presence of the reference furanone compound (Fur) or new furanone compounds (**QS-D: 13**, **15**, and **17**) at a concentration of 2 μM. Bacterial growth was monitored by measuring absorbance at 600 nm using a spectrophotometer.

2.4 Cytotoxicity evaluation

A cytotoxicity test demonstrated that the compounds did not affect host cell viability at 2 μM as shown in Fig. 5. The experiments were carried out in three types of human cells: the monocytic cell line THP-1, human gingival fibroblasts (HGFs), and the human oral keratinocyte cell line HOK-16B.

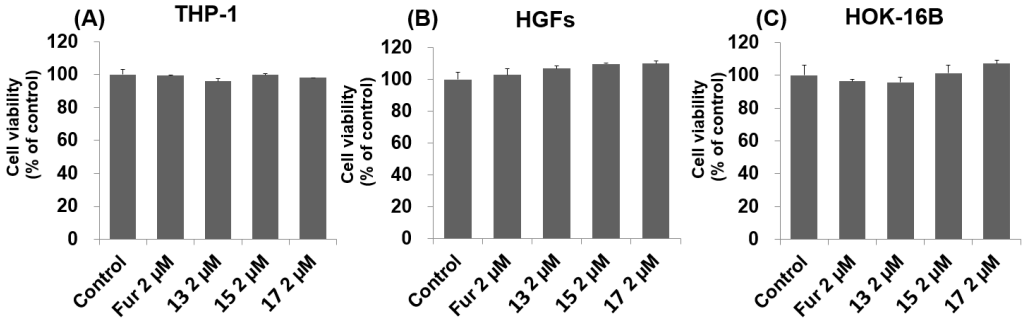


Fig. 5. Effects of furanone compounds on host cell viability. (A) THP-1 cells (1×10^5 cells/well), (B) HGFs (2×10^4 cells /well), and (C) HOK-16B cells (5×10^4 cells/well) cultured in 96-well plates were treated with the reference furanone compound (Fur) and new furanone compounds (**QS-D: 13**, **15**, and **17**) for 24 h, and the cell viability was detected using Cell Counting Kit-8. The control was the non-treated group. The experiments were performed three times in triplicate and representative data are shown.

2.5 Effect on the host cell immune response

The expression levels of proinflammatory cytokines, including interleukin (IL)-6 and IL-8, were tested to evaluate whether the new compounds induce an inflammatory response in host cells. They were analyzed by real-time reverse transcription-polymerase chain reaction (RT-PCR) after treating THP-1 cells and HGFs with the furanone compounds. As shown in Fig. 6, both in THP-1 and HGFs, the new furanone compounds (**QS-D: 13**, **15**, and **17**) did not induce an

inflammatory response, whereas the reference furanone or lipopolysaccharide (LPS; 1 μg/mL) significantly induced the gene expression of IL-6 and IL-8.

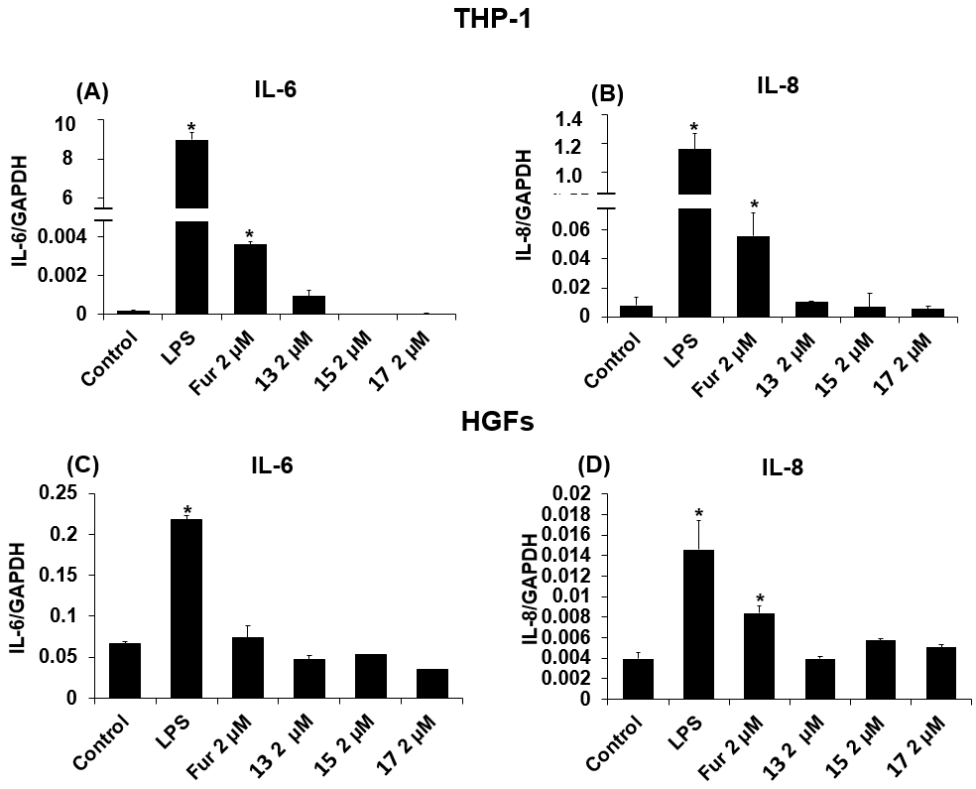


Fig. 6. Effects of furanone compounds on proinflammatory cytokines. THP-1 cells (1×10^6 cells/well, A and B) and HGFs (2×10^5 cells /well, C and D) cultured in 6-well plates were treated with the reference furanone compound (Fur) and new furanone compounds (**QS-D: 13**, **15**, and **17**) for 24 h. The expression levels of *IL-6* and *IL-8* mRNA were analyzed by real-time RT-PCR. The experiments were performed three times in triplicate, and representative data are shown. $*p < 0.05$ compared to the untreated control value.

3. Conclusion

We synthesized bicyclic diriminated furanone derivatives and tested their biological inhibitory effects. Among these compounds tested, the one containing a

five-membered ring (**QS-D:13**) and the ones have methylbenzene moieties (**QS-D: 15** and **17**) showed the best activities. They significantly inhibited the *F. nucleatum* AI-2 activity and biofilm formation of *F. nucleatum*, *P. gingivalis,* and *T. forsythia* without disturbing bacterial growth. The inhibitory activities of **QS-D: 13**, **15**, and **17** were similar to or better than that of the reference furanone. In addition, our new furanone compounds showed neither cytotoxicity nor induction of proinflammatory cytokine expression, whereas the reference furanone compound showed cytotoxicity and induced the expression of proinflammatory cytokines such as IL-6 and IL-8 in human cells.

Our results highlight the importance of the bicyclic structure for its effective biofilm inhibition activity. With emerging issues, such as antibacterial multidrug resistance, development of new therapeutic methods is being actively pursued all over the world. Inhibition of QS has become one of the most promising therapeutic approaches for disease control. In this study, it showed that the new furanone compounds **QS-D: 13**, **15**, and **17** can be good candidates for preventing periodontitis.

4. Experimental procedures

The ^{1}H, ^{13}C NMR-spectra and heteronuclear multiple bond correlation (HMBC) were measured with an Agilent 400-MR DD2 Magnetic Resonance System (400 MHz) and a Varian/Oxford As-500 (500 MHz) spectrophotometer. Chemical shifts were measured as parts per million (δ values) from tetramethylsilane as an internal standard at probe temperature in $CDCl_3$ or DMSO-D_6 for neutral compounds. Coupling constants are provided in Hz, with the following spectral pattern designations: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; br, broad; app, apparent. Reactions that needed anhydrous conditions were carried

out in flame-dried glassware under positive pressure of dry N_2 using standard Schlenk line techniques. Evaporation of solvents was performed at reduced pressure using a rotary evaporator. TLC was performed using silica gel 60F254 coated on aluminum sheet (E. Merck, Art.5554). Column chromatography was performed on silica gel (Merck. 7734 or 9385 Kiesel gel 60), and eluent was mentioned in each procedure. High resolution mass spectra (HRMS) were recorded on a ThermoFinnigan LCQ™ Classic, Quadrupole Ion-Trap Mass Spectrometer. HPLC analyses were carried out on an Agilent HP1100 system (Santa Clara, CA, USA), composed of an auto sampler, quaternary pump, photodiode array detector (DAD), and HP Chemstation software. The separation was arried out on a poroshell 120 EC-C18 column 4.6 × 50 mm i.d. (2.7 m particle size) with 0.1% TFA in water (A) and acetonitrile (B) as a mobile phase at a flow rate of 1 mL/min at 20 °C. Method: 100% A and 0% B (0 min), 50% A and 50% B (5 min), 5% A and 95% B (15 min), 5% A and 95% B (22 min), 100% A and 0% B (23 min), 100% A and 0% B (25 min). Details are given below for one example of each reaction type; the syntheses and characterization of analogues are reported in the Supplementary Information. All materials were obtained from commercial supplier and used without further purification unless otherwise noted.

*4.1. General procedure for the preparation of 3 or 4-alkoxyphthalic anhydride (**QS-D: 7a–7e, 9a–9d**)*

Following a reported procedure [26], add H_2SO_4 (0.054 mL, 1.02 mmol) to a stirred solution of 3- or 4-hydroxyphthalic acid (1.09 g, 6.00 mmol) in 12 mL of MeOH. The reaction was stirred at reflux overnight. Solvent was removed under reduced pressure and the solid residue obtained was dissolved in dichloromethane (40 mL) and washed with water (20 × 3 mL). The combined organic layer was dried

over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to provide crude dimethyl 4-hydroxyphthalate. The crude dimethyl 3- or 4-hydroxyphthalate was dissolved in 3 mL of acetone and K_2CO_3 (1.3 g, 9.5 mmol) was added to the solution. The mixture was stirred at room temperature for 1 h. Alkyl iodide (5.7 mmol) was added, and the mixture was stirred at reflux overnight. and the solvent was removed under reduced pressure to provide dimethyl 3- or 4-alkoxyphthalate.

A solution of dimethyl 3- or 4-alkoxyphthalate in 3 mL of acetone was treated with an aq solution of NaOH (0.34 g, 8.4 mmol) in 2 mL water and then the resulting solution was stirred at room temperature overnight. After evaporation of the solvent, the mixture was acidified with 6 M HCl to pH 2 and concentrated under reduced pressure. Then, the 3- or 4-alkoxyphthalic acid was dissolved in 4 mL of Ac_2O and the solution was heated at reflux for 18 h. Upon cooling to room temperature, the resulting solution was concentrated by rotary evaporation and then the residue was purified with flash column chromatography. The desired products **QS-D: 7a-7e** and **9a–9d** were obtained.

*4.1.1. 4-Methoxyphthalic anhydride (**QS-D: 7a**).* Yield 0.80 g (75%). ^{1}H NMR (DMSO-d6, 500 MHz) δ 7.99 (d, J = 8.4 Hz, 1H), 7.58 (d, J = 2.3 Hz, 1H), 7.48 (dd, J = 8.5, 2.3 Hz, 1H), 3.97 (s, 3H). ^{13}C NMR (DMSO-d6, 125 MHz) δ 165.7, 163.1, 162.7, 134.0, 127.2, 122.9, 122.7, 109.3, 56.7.

*4.1.2. 4-Ethoxyphthalic anhydride (**QS-D: 7b**).* Yield 0.83 g (72%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.96 (d, *J* = 8.4 Hz, 1H), 7.53 (d, *J* = 2.3 Hz, 1H), 7.45 (dd, *J* = 8.4, 2.3 Hz, 1H), 4.25 (q, *J* = 7.0 Hz, 2H), 1.37 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 164.92, 163.1, 162.6, 134.0, 127.2, 123.1, 122.5, 109.6,

64.9, 14.3.

*4.1.3. 4-Propoxyphthalic anhydride (**QS-D: 7c**)*. Yield 0.86 g (70%). ^{1}H NMR (DMSO-d6, 500 MHz) δ 7.97 (d, J = 8.4 Hz, 1H), 7.55 (d, J = 2.2 Hz, 1H), 7.47 (dd, J = 8.4, 2.3 Hz, 1H), 4.16 (t, J = 6.5 Hz, 2H), 1.81 – 1.72 (m, 2H), 0.99 (t, J = 7.4 Hz, 3H). 13C NMR (DMSO-d6, 125 MHz) δ 165.1, 163.1, 162.7, 134.0, 127.2, 123.1, 122.5, 109.6, 70.5, 21.7, 10.2.

*4.1.4. 4-Butoxyphthalic anhydride (**QS-D: 7d**)*. Yield 0.97 g (74%). ^{1}H NMR (DMSO-d6, 500 MHz) δ 7.97 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 2.3 Hz, 1H), 7.47 (dd, J = 8.5, 2.2 Hz, 1H), 4.20 (t, J = 6.5 Hz, 2H), 1.79 – 1.68 (m, 2H), 1.51 – 1.38 (m, 2H), 0.94 (t, J = 7.4 Hz, 3H). 13C NMR (DMSO-d6, 125 MHz) δ 165.1, 163.1, 162.7, 134.0, 127.2, 123.2, 122.5, 109.6, 68.8, 30.3, 18.6, 13.6.

*4.1.5. 4-Pentyloxyphthalic anhydride (**QS-D: 7e**)*. Yield 0.98 g (70%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.96 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 2.2 Hz, 1H), 7.46 (dd, *J* = 8.4, 1.8 Hz, 1H), 4.19 (t, *J* = 6.5 Hz, 2H), 1.82 – 1.69 (m, 2H), 1.47 – 1.28 (m, 4H), 0.90 (t, *J* = 7.1 Hz, 3H).

*4.1.6. 3-Ethoxyphthalic anhydride (**QS-D: 9a**)*. Yield 0.61 g (53%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.92 (dd, *J* = 8.5, 7.4 Hz, 1H), 7.58 (dd, *J* = 16.4, 7.9 Hz, 2H), 4.31 (q, *J* = 7.0 Hz, 2H), 1.40 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 163.2, 160.6, 156.9, 138.7, 133.0, 120.2, 116.8, 116.4, 65.0, 14.3.

*4.1.7. 3-Propoxyphthalic anhydride (**QS-D: 9b**)*. Yield 0.58 g (47%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.92 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.56 (d, *J* = 7.4 Hz, 1H), 4.20 (t, *J* = 6.4 Hz, 2H), 1.85 – 1.75 (m, 2H), 1.02 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 163.2, 160.6, 157.1, 138.7, 133.0, 120.3, 116.8, 116.4, 70.5, 21.7, 10.1.

*4.1.8. 3-Butoxyphthalic anhydride (**QS-D: 9c**)*. Yield 0.59 g (45%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.91 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.56 (d, *J* = 7.3 Hz, 1H), 4.24 (t, *J* = 6.4 Hz, 2H), 1.82 – 1.71 (m, 2H), 1.54 – 1.42 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 163.2, 160.5, 157.1, 138.7, 133.0, 120.2, 116.8, 116.4, 68.8, 30.3, 18.5, 13.6.

*4.1.9 3-Pentyloxyphthalic anhydride (**QS-D: 9d**)*. Yield 0.64 g (46%). ^{1}H NMR (DMSO-d6, 500 MHz) δ 7.91 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 7.4 Hz, 1H), 4.23 (t, *J* = 6.5 Hz, 2H), 1.79 (dd, *J* = 14.1, 7.3 Hz, 2H), 1.52 – 1.30 (m, 4H), 0.90 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 163.2, 160.5, 157.1, 138.7, 133.0, 120.2, 116.8, 116.4, 69.1, 28.0, 27.4, 21.8, 13.9.

*4.2. General procedure for the preparation of bicyclic brominated furanone derivatives **(QS-D: 10– 18d)***

Triphenylphosphine (1.60 g, 6.00 mmol) was dissolved in 6 mL of THF and the solution was cooled to 0 °C under Ar. A solution of CBr_4 (1.00 g, 3.00 mmol) in THF (1.00 mL per mmol PPh_3) was added to the THF (6 mL) solution of PPh_3 and the reaction was stirred until the color changed to yellow. Triethylamine (TEA) (1.1 mL, 6.0 mmol) was added dropwise to the mixture over 5 min, then a solution of an anhydride compound (**QS-D: 1–9d**, 1.00 mmol) in THF (1.0 mL per mmol phthalic anhydride) was added. The reaction was stirred for 1 h at 0 °C, after which it was allowed to warm to room temperature and stirred overnight. The reaction was quenched by addition of sat aq. NH_4Cl solution (3 mL). The phases were then separated, and the crude product was extracted with hexane from the aqueous layer (3 × 30 mL). The combined organic layers were concentrated under reduced

pressure. The residue was dissolved in Et_2O (25 mL) and filtered over a Celite pad (7.0 × 3.0 cm). The resulting filtrate was concentrated by rotary evaporation and the crude products were purified through the column chromatography (5–15% EtOAc in hexane).

*4.2.1 3-(Dibromomethylene)isobenzofuran-1(3H)-one (**QS-D: 10**).* Yield 0.091 g (30%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.32 (d, *J* = 8.0 Hz, 1H, H-4), 7.95 (d, *J* = 7.6 Hz, 1H, H-7), 7.90 (t, *J* = 7.7 Hz, 1H, H-5), 7.75 (t, *J* = 7.5 Hz, 1H, H-6). ^{13}C NMR (DMSO-d_6,125 MHz) δ 164.0 (C-1), 145.5 (C-3), 135.8 (C-3a), 135.6 (C-5), 131.4 (C-6), 126.0 (C-7), 125.3 (C-7a), 123.7 (C-4), 77.3 (=CBr_2). HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_9H_4Br_2NaO_2$+: 324.8470; found 324.8471.

*4.2.2 3-(Dibromomethylene)hexahydroisobenzofuran-1(3H)-one (**QS-D:11**).* Yield 0.12 g (40%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.31 – 3.19 (m, 1H), 2.98 (t, *J* = 6.8 Hz, 1H), 2.22 (t, *J* = 14.3 Hz, 2H), 1.72 (d, *J* = 15.0 Hz, 2H), 1.35 – 1.05 (m, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.1, 158.2, 68.9, 40.7, 40.0, 26.5, 23.0, 22.3. HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_9H_{10}Br_2NaO_2$+: 330.8940; found 330.8942.

*4.2.3 3-(Dibromomethylene)-4,5,6,7-tetrahydroisobenzofuran-1(3H)-one (**QS-D 12**).* Yield 0.20 g (65%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.74 – 2.69 (m, 2H), 2.16 – 2.11 (m, 2H), 1.72 – 1.68 (m, 2H), 1.63 – 1.59 (m, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 166.5, 149.7, 148.6, 131.2, 78.4, 25.0, 21.3, 20.5, 20.1. HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_9H_8Br_2NaO_2$+: 328.8783; found 328.8786.

*4.2.4. 3-(Dibromomethylene)hexahydro-1H-cyclopenta[c]furan-1-one (**QS-D 13**).* Yield 0.071 g (24%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.50 – 3.48 (m, 2H), 2.04 – 1.82 (m, 4H), 1.73 – 1.66 (m, 1H), 1.39 – 1.30 (m, 1H). ^{13}C NMR (DMSO-d_6, 125

MHz) δ 176.5, 155.4, 68.0, 45.8, 43.8, 32.2, 30.8, 25.6. HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_8H_8Br_2NaO_2$+: 316.8783; found 316.8780.

*4.2.5. 3-(Dibromomethylene)-3,4,5,6-tetrahydro-1H-cyclopenta[c]furan-1-one (**QS-D: 14**).* Yield 0.080 g (27%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.96 – 2.91 (m, 2H), 2.48 – 2.39 (m, 4H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 162.5, 162.2, 146.5, 140.9, 79.4, 29.5, 28.1, 25.1. HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_8H_6Br_2NaO_2$+: 314.8627; found 314.8625.

*4.2.6. 3-(Dibromomethylene)-5-methylisobenzofuran-1(3H)-one (**QS-D:15**).* Yield 0.076 g (24%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.18 (s, 1H, H-4), 7.87 (d, *J* = 7.9 Hz, 1H, H-7), 7.60 (d, *J* = 7.8 Hz, 1H, H-6), 2.52 (s, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 164.0 (C-1), 146.9 (C-5), 145.5 (C-3), 136.3 (C-3a), 132.6 (C-6), 126.0 (C-7), 124.0 (C-4), 122.9 (C-7a), 77.0 (=CBr_2), 22.0 (-CH_3). HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_{10}H_6Br_2NaO_2$+: 338.8627; found 338.8629.

*4.2.7. 3-(Dibromomethylene)-5-methoxyisobenzofuran-1(3H)-one (**QS-D:16a**).* Yield 0.073 g (22%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.91 (d, *J* = 8.5 Hz, 1H, H-7), 7.76 (s, 1H, H-4), 7.32 (d, *J* = 7.0 Hz, 1H, H-6), 3.93 (s, 3H, -OCH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 165.0 (C-5), 163.5 (C-1), 145.1 (C-3), 138.2 (C-3a), 127.9 (C-7), 118.2 (C-6), 117.5 (C-7a), 108.2 (C-4), 77.4 (=CBr_2), 56.2 (-OCH_3). HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_{10}H_6Br_2NaO_3$+: 354.8576; found 354.8576.

*4.2.8. 3-(Dibromomethylene)-5-ethoxyisobenzofuran-1(3H)-one (**QS-D:16b**).* Yield 0.70 g (20%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.90 (d, *J* = 8.5 Hz, 1H, H-7), 7.76 (s, 1H, H-4), 7.31 (d, *J* = 7.0 Hz, 1H, H-6), 4.20 (q, *J* = 6.8 Hz, 2H, -OCH_2), 1.39 (t, *J* = 6.9 Hz, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 164.3 (C-5), 163.6 (C-1), 145.2 (C-3), 138.2 (C-3a), 128.0 (C-7), 118.4 (C-6), 117.3 (C-7a), 108.7 (C-

4), 77.4 (=CBr_2), 64.6 (-OCH_2), 14.3 (-CH_3). HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_{11}H_8Br_2NaO_3$+: 368.8732; found 368.8731.

*4.2.9. 3-(Dibromomethylene)-5-propoxyisobenzofuran-1(3H)-one (**QS-D:16c**).* Yield 0.69 g (19%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.89 (d, *J* = 8.5 Hz, 1H, H-7), 7.75 (s, 1H, H-4), 7.31 (d, *J* = 8.5 Hz, 1H, H-6), 4.09 (t, *J* = 6.4 Hz, 2H, -OCH_2), 1.84 – 1.75 (m, 2H, -CH_2), 1.00 (t, *J* = 7.4 Hz, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 164.4 (C-5), 163.5 (C-1), 145.1 (C-3), 138.1 (C-3a), 127.9 (C-7), 118.5 (C-6), 117.3 (C-7a), 108.7 (C-4), 77.4 (=CBr_2), 70.2 (-OCH_2), 21.8(-CH_2), 10.3(-CH_3). HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_{12}H_{10}Br_2NaO_3$+: 382.8889; found 382.8887.

*4.2.10. 3-(Dibromomethylene)-5-butoxyisobenzofuran-1(3H)-one (**QS-D:16d**).* Yield 0.79 g (21%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.90 (d, *J* = 8.5 Hz, 1H, H-7), 7.76 (s, 1H, H-4), 7.31 (d, *J* = 8.5 Hz, 1H, H-6), 4.14 (t, *J* = 6.4 Hz, 2H, -OCH_2), 1.80 – 1.68 (m, 2H, -CH_2), 1.50 – 1.39 (m, 2H, -CH_2), 0.94 (t, *J* = 7.4 Hz, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 164.4 (C-5), 163.5 (C-1), 145.2 (C-3), 138.2 (C-3a), 128.0 (C-7), 118.4 (C-6), 117.3 (C-7a), 108.7 (C-4), 77.4 (=CBr_2), 68.4 (-OCH_2), 39.5 (-CH_2), 30.4 (-CH_2), 18.6 (-CH_2), 13.6 (-CH_3). HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_{13}H_{12}Br_2NaO_3$+: 396.9045; found 396.9047.

*4.2.11. 3-(Dibromomethylene)-5-(pentyloxy)isobenzofuran-1(3H)-one (**QS-D 16e**).* Yield 0.74 g (19%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.88 (d, *J* = 8.5 Hz, 1H, H-7), 7.74 (s, 1H, H-4), 7.30 (d, *J* = 8.6 Hz, 1H, H-6), 4.12 (t, *J* = 6.4 Hz, 2H, -OCH_2), 1.81 – 1.72 (m, 2H, -CH_2), 1.46 – 1.29 (m, 4H, -CH_2), 0.90 (t, *J* = 7.1 Hz, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 164.4 (C-5), 163.5 (C-1), 145.1 (C-3), 138.1 (C-3a), 127.9 (C-7), 118.4 (C-6), 117.3 (C-7a), 108.7 (C-4), 77.4 (=CBr_2), 68.7 (-CH_2), 28.1 (-CH_2), 27.5 (-CH_2), 21.8 (-CH_2), 13.9(-CH_3). HRMS (ESI) m/z:

Anal. calcd. for [M+Na]+ $C_{14}H_{14}Br_2NaO_3$+: 410.9202; found 410.9203.

*4.2.12. 3-(Dibromomethylene)-7-methylisobenzofuran-1(3H)-one (**QS-D:17**).* Yield 0.83 g (26%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.18 (d, *J* = 7.9 Hz, 1H, H-4), 7.77 (t, *J* = 7.7 Hz, 1H, H-5), 7.55 (d, *J* = 7.5 Hz, 1H, H-6), 2.61 (s, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 164.0 (C-1), 145.2 (C-3), 139.8 (C-7), 136.1 (C-3a), 135.2 (C-5), 132.9 (C-6), 122.8 (C-7a), 121.3 (C-4), 76.5 (=CBr_2), 16.9 (-CH_3). HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_{10}H_6Br_2NaO_2$+: 338.8627; found 338.8628.

*4.2.13. 3-(Dibromomethylene)-7-ethoxyisobenzofuran-1(3H)-one (**QS-D: 18a**).* Yield 0.66 g (19%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.87 (d, *J* = 7.8 Hz, 1H, H-4), 7.82 (t, *J* = 8.0 Hz, 1H, H-5), 7.32 (d, *J* = 8.2 Hz, 1H, H-6), 4.24 (q, *J* = 6.9 Hz, 2H, -OCH_2), 1.38 (t, *J* = 7.0 Hz, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.4 (C-1), 157.8 (C-7), 145.1 (C-3), 137.8 (C-5), 137.7 (C-3a), 115.3 (C-4), 114.7 (C-6), 111.8 (C-7a), 76.7 (=CBr_2), 64.6 (-OCH_2), 14.3 (-CH_3). HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_{11}H_8Br_2NaO_3$+: 368.8732; found 368.8733.

*4.2.14. 3-(Dibromomethylene)-7-propoxyisobenzofuran-1(3H)-one (**QS-D: 18b**).* Yield 0.65 g (18%). %). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.87 (d, *J* = 7.8 Hz, 1H, H-4), 7.82 (t, *J* = 8.0 Hz, 1H, H-5), 7.32 (d, *J* = 8.3 Hz, 1H, H-6), 4.13 (t, *J* = 6.4 Hz, 2H, -OCH_2), 1.85 – 1.73 (m, 2H, -CH_2), 1.01 (t, *J* = 7.4 Hz, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.4 (C-1), 157.9 (C-7), 145.1 (C-3), 137.8 (C-5), 137.7 (C-3a), 115.4 (C-4), 114.8 (C-6), 111.9 (C-7a), 76.7 (=CBr_2), 70.1 (-OCH_2), 21.8 (-CH_2), 10.2 (-CH_3). HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_{12}H_{10}Br_2NaO_3$+: 382.8889; found 382.8891.

*4.2.15. 3-(Dibromomethylene)- 7-butoxyisobenzofuran-1(3H)-one (**QS-D: 18c**).*

Yield 0.68 g (18%). ^{1}H NMR (DMSO-d6, 500 MHz) δ 7.88 (d, *J* = 7.8 Hz, 1H, H-4), 7.82 (t, *J* = 8.0 Hz, 1H, H-5), 7.33 (d, *J* = 8.3 Hz, 1H, H-6), 4.18 (t, *J* = 6.4 Hz, 2H, -OCH_2), 1.80 – 1.66 (m, 2H, -CH_2), 1.53 – 1.37 (m, 2H, -CH_2), 0.94 (t, *J* = 7.4 Hz, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.4 (C-1), 157.9 (C-7), 145.1 (C-3), 137.9 (C-5), 137.7 (C-3a), 115.4 (C-4), 114.8 (C-6), 111.9 (C-7a), 76.7 (=CBr_2), 68.5 (-CH_2), 30.4 (-CH_2), 18.6 (-CH_2), 13.7 (-CH_3). HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_{13}H_{12}Br_2NaO_3$+: 396.9045; found 396.9047.

*4.2.16. 3-(Dibromomethylene)-7-(pentyloxy)isobenzofuran-1(3H)-one (**QS-D: 18d**).* Yield 0.62 g (16%) ^{1}H NMR (DMSO-d6, 500 MHz) δ 7.89 (d, *J* = 7.8 Hz, 1H, H-4), 7.83 (t, *J* = 8.0 Hz, 1H, H-5), 7.34 (d, *J* = 8.3 Hz, 1H, H-6), 4.18 (t, *J* = 6.5 Hz, 2H, -OCH_2), 1.84 – 1.70 (m, 2H, -CH_2), 1.50 – 1.28 (m, 4H, -CH_2CH_2), 0.89 (t, *J* = 7.2 Hz, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.4 (C-1), 157.9 (C-7), 145.1 (C-3), 137.9 (C-5), 137.7 (C-3a), 115.4 (C-4), 114.8 (C-6), 111.9 (C-7a), 76.6 (=CBr_2), 68.8 (-CH_2), 28.0 (-CH_2), 27.5 (-CH_2), 21.8 (-CH_2), 13.9 (-CH_3). HRMS (ESI) m/z: Anal. calcd. for [M+Na]+ $C_{14}H_{14}Br_2NaO_3$+: 410.9202; found 410.9201.

II. Bicyclic monobrominated furanones

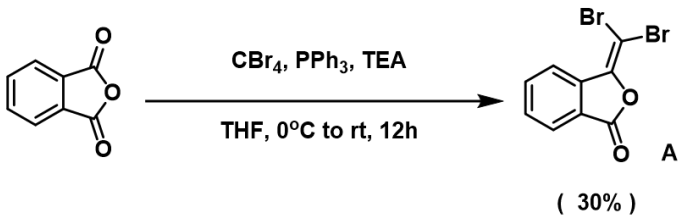
1. Chemistry

We continued to study a series of monobrominated furanone derivatives, which were expected to have biofilm inhibitory activities. All of them were synthesized by only two steps. Although the first step, gem-dibromoolefination of phthalic anhydride, which was carried out according to our previous method, gave the expected product, nevertheless the yields were not appreciably high. Therefore, optimization was done first in this work. Inspired by the work of Y.-Q. Fang et

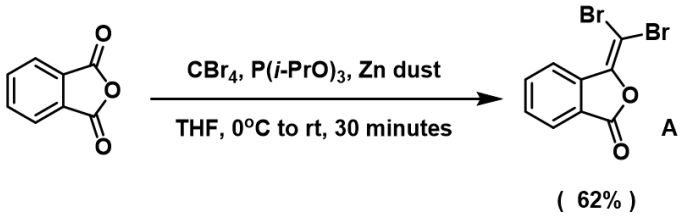
al.[27], replacing triphenylphosphine with triisopropyl phosphite, not only reduced the waste of reagents but also increased yields. After a series of experiments, we found that with the addition of zinc dust in THF, the reaction were complete in only 30 minutes with satisfactory yields of the desired products.

Scheme 2: **gem-dibromoolefination of phthalic anhydride.**

Previous work[a]



This work[b]



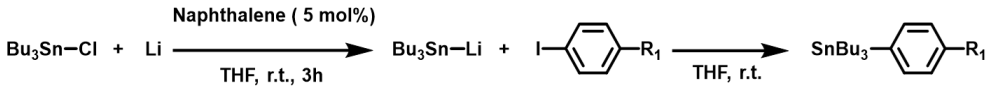
a. Phthalic anhydride (1.0 mmol), CBr_4 (3.0 mmol), PPh_3 (6.0 mmol), TEA (6.0 mmol). b. Phthalic anhydride (1.0 mmol), CBr_4 (1.5 mmol), $P(i\text{-}PrO)_3$ (2.2 mmol), Zn dust (1.5 mmol)

Table 3: **Optimization of reaction conditions**

Entry	Reagent	Solvent	Cat.	Base	temperature	Time	Yield[a]
1	PPh_3	THF		TEA	0°C to rt.	12 h	30 %
2	PPh_3	THF		DIPEA	0°C to rt.	12 h	27 %
3	P(O*i*-Pr)$_3$	THF			0°C to rt.	1 h	45 %
4	P(O*i*-Pr)$_3$	THF			- 10 °C	12 h	28 %
5[b]	P(O*i*-Pr)$_3$	DCM			0°C to rt.	24 h	
6[c]	P(O*i*-Pr)$_3$	DME			0°C to rt.	24 h	
7	P(O*i*-Pr)$_3$	THF	DMAP		0°C to rt.	30 minutes	38 %
8	P(O*i*-Pr)$_3$	THF	Zn dust		0°C to rt.	30 minutes	62%

a. yields of isolated products. b. incomplete conversion, yield <15 %.c. incomplete conversion, 40% yield <19%.

Scheme 3: Synthesis of tin compounds.



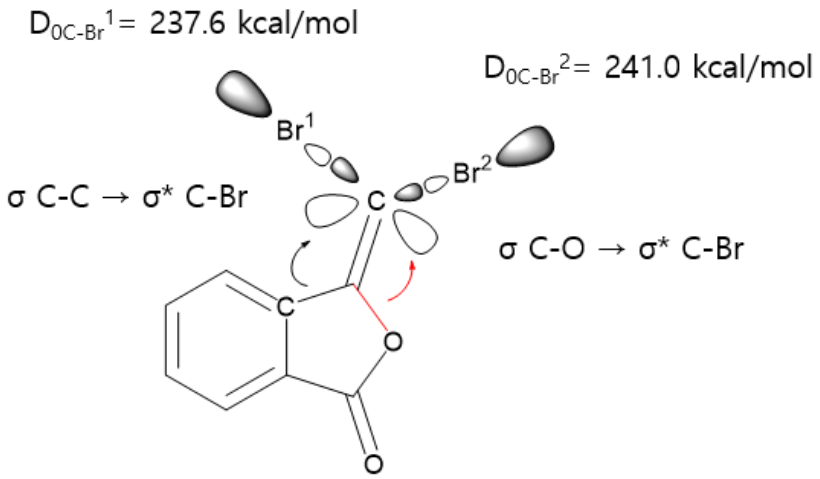
R_1 = F, CF_3

Then monobrominated furanone derivatives were all obtained through Stille coupling reaction. Tin reagents from entries 5 and 6 were prepared according to the method reported by D.Y. Wang et al. [28], and the others were all obtained from commercial supplier and used as received. The upfield chemical shift of ^{1}H of the aromatic ring next to the olefin demonstrates that Z-form isomer is the major product. And only Z-form product was found in some reactions as shown below in Table 3 (entries 1-6, and 10). The structure of compound **QS-M 1** was also

confirmed by single-crystal X-ray diffraction experiment (Fig 8). Achim Sorg et al. and Vladislav Kotek et al. also obtained Z-form isomers as major products in their work [29–31].

We believe that regioselectivity occurs during oxidative addition step (Scheme 4). To understand this, notice that σC-O is a better donor than σC-C, which can make C-Br^1 bond longer than C-Br^2 bond. Additionally, we calculated the bond-dissociation energy (D_0) of C-Br^1 and C-Br^2 (Fig 8) and found that C-Br^1 bond is weaker than C-Br^2 bond. This lends further credence to our belief. All the computational calculations in this work were performed using the Gaussian 09 program package with B3LYP hybrid functional. The 6-31G(d) basis set was used for all atoms.

Fig 7: σ C-O is a better donor than σ C-C



Scheme 4: Mechanism of Stille coupling

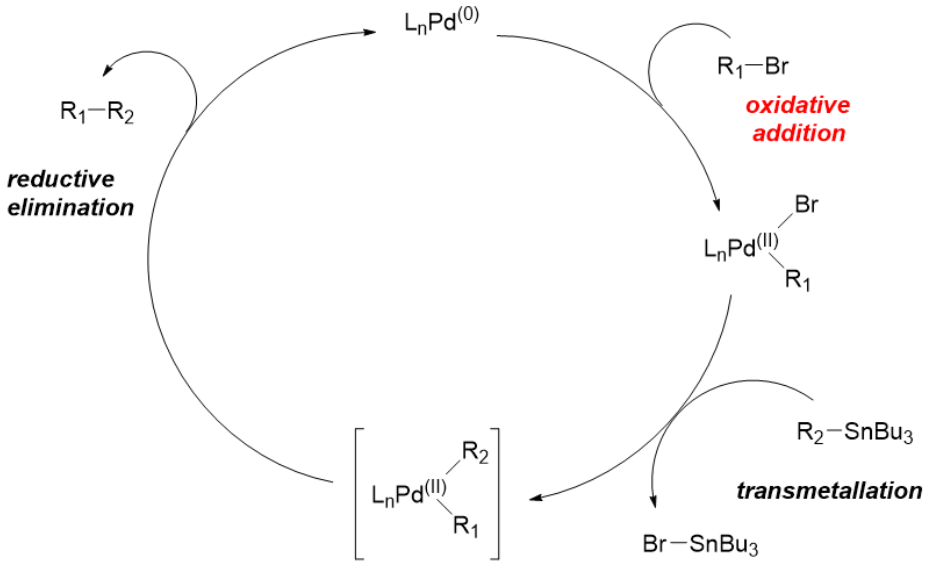


Fig 8: Single crystal X-ray diffraction of 1

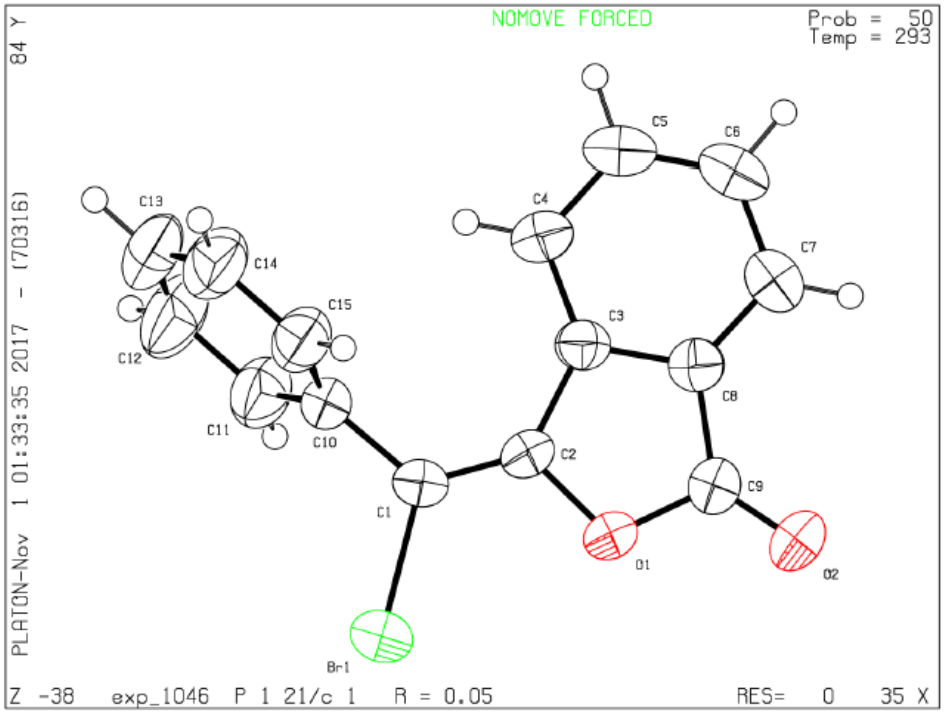
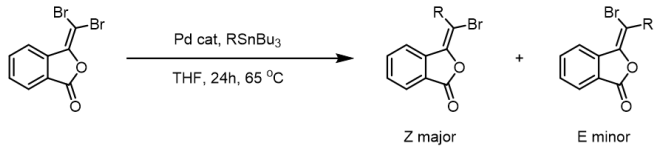
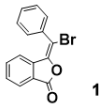
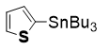
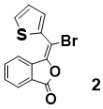
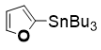
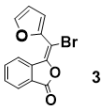
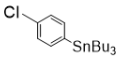
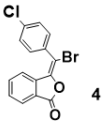
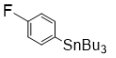
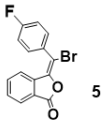
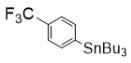
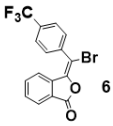
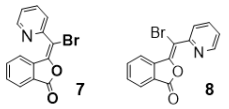
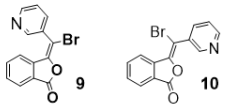
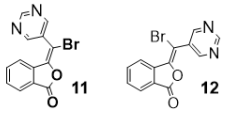
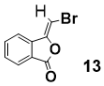


Table 4: **Synthesis of monobrominated furanone derivatives (QS-M)**



Entry	Reagent	Cat.	Ligand	Product	Yield[a]
1		$Pd(dba)_2$	$AsPh_3$	 **1**	72%
2		$Pd(dba)_2$	$AsPh_3$	 **2**	65%
3		$Pd(dba)_2$	$AsPh_3$	 **3**	62%
4		$Pd(dba)_2$	$AsPh_3$	 **4**	59%
5		$Pd(dba)_2$	$AsPh_3$	 **5**	75%
6		$Pd(dba)_2$	$AsPh_3$	 **6**	67%
7		$Pd(dba)_2$	$AsPh_3$	 **7** **8**	80% (Z:E=3:2)
8		$Pd(dba)_2$	$AsPh_3$	 **9** **10**	45% (Z:E=5:2)
9		$Pd(dba)_2$	$AsPh_3$	 **11** **12**	53% (Z:E=6:1)
10	$HSnBu_3$	$Pd(pph_3)_4$		 **13**	25%

a. Yields of isolated products

2. Biological assays

The Compound **QS-M 1**, which was confirmed by single-crystal X-ray diffraction, was first tested for its inhibitory activity in comparison with the reference furanone R1, QS-131 and QS-106 (which have highest activities in our previous work). As shown in Fig 8, compound **QS-M 1** (QLZ) also showed significant inhibitory activity.

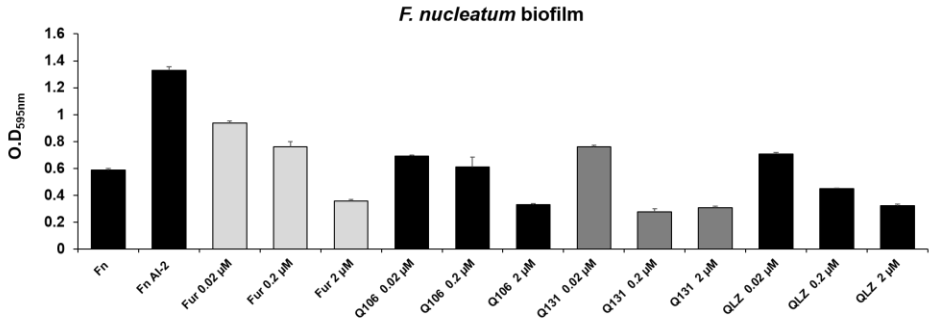


Fig 9. Inhibitory effects of 1 on the biofilm formation of periodontopathogens at various concentrations.

	Biofilm formation (% of control)		
	0.02 μM	0.2 μM	2 μM
Fn AI-2	100.00	100.00	100.00
Furanone	73.04	60.87	33.14
Q106	56.29	50.62	31.40
Q131	60.87	27.64	29.75
QS-LLZ	57.21	39.68	30.76

Table 5: Biofilm formation (% of control) of 1(QS-LLZ) comparing with furanone, Q106 and Q131

All the monobrominated furanones (**QS-M: 1-13**) were screened for their inhibitory effects on the biofilm formation of F. *nucleatum* at 0.2 μM concentrations together with R1. Among these compounds, **QS-M: 1** and **11** showed better inhibitory activities than all of the others. And continuous biological assays are in progress.

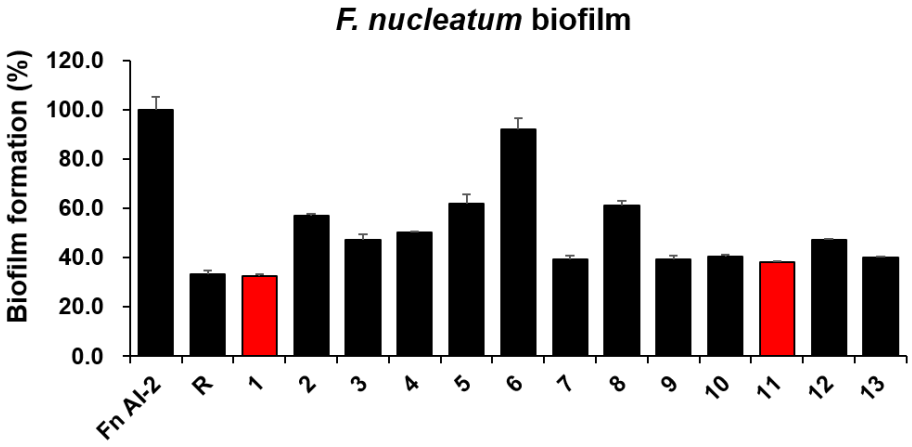


Fig. 10. Biofilm formation (% of control) of 1-13 in comparison with R1

#	Biofilm formation (%)
Fn Al-2	100
R	33.26418
1	32.57261
2	56.98479
3	47.37206
4	50.20747
5	61.75657
6	92.04703
7	39.34993
8	60.99585
9	39.14246
10	40.45643
11	38.10512
12	47.16459
13	39.90318

Table 6. Biofilm formation (% of control) of 1-13 comparing with R

3. Conclusion

We designed and synthesized a series of monobrominated isobenzofuranone derivatives. All of them can be synthesized simply in two steps. The optimization of the first step, gem-dibromoolefination of phthalic anhydride, was done by replacing PPh_3 with $P(O\textit{i}\text{-}Pr)_3$ and adding Zn dust. After Stille coupling of this dibrominated furanone (A), a Z form isomer was obtained as a major product. This is confirmed by ^1H NMR and also single crystal X-ray diffraction.

These compounds were screened for their inhibitory effects on the biofilm formation of F. nucleatum at 0.2 μM concentrations in reference with R1. Among these compounds, **QS-M: 1** and **11** showed most pronounced inhibitory activities.

4. Experimental procedures

The ^1H, ^{13}C NMR-spectra were measured with an Agilent 400-MR DD2 Magnetic Resonance System (400 MHz) spectrophotometer and a Varian/Oxford As-500 (500 MHz) spectrophotometer. Chemical shifts were measured as part per million (δ values) from tetramethylsilane(TMS) as an internal standard at probe temperature in DMSO-D6 for neutral compounds. Coupling constants are provided in Hz, with the following spectral pattern designations: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; br, broad; app, apparent. Reactions that needed anhydrous conditions were carried out in flame-dried glassware under positive pressure of dry Ar using standard Schlenk line techniques. Evaporation of solvents was performed at reduced pressure using a rotary evaporator. TLC was performed using silica gel 60F254 coated on aluminum sheet (E. Merck, Art.5554). Column chromatography was performed on silica gel (Merck. 7734 or 9385 Kiesel gel 60). High resolution mass spectra (HRMS) were recorded on a ThermoFinnigan LCQ™ Classic, Quadrupole Ion-Trap Mass Spectrometer. HPLC analyses were

carried out on an Agilent HP1100 system (Santa Clara, CA, USA), composed of an auto sampler, quaternary pump, photodiode array detector (DAD), and HP Chemstation software. Phthalic anhydride was used after recrystallization and Tetrabromomethane was used after dried over MgSO4. All materials were obtained from commercial supplier.

*4.1. General procedure for the preparation of (**A**)*

Triisopropylphosphite (2.7 ml, 5.00 mmol) in 10 mL of THF with Zn dust (490 mg, 7.50 mmol) was cooled to 0 °C under Ar. A solution of CBr_4 (2.49 g, 7.50 mmol) in THF was added to the solution of $P(Oi\text{-}Pr)_3$ and the reaction was stirred until the color changed to yellow. Then a solution of phthalic anhyride in THF was added. The reaction was stirred for 30 minutes at room temperature. After quenched by addition of sat aq $NaHCO_3$ solution (2.5 mL), The phases were then separated, and the crude product was extracted with either from the aqueous layer. The residue was dissolved in either and filtered over a Celite pad. The resulting filtrate was concentrated by rotary evaporation and the desired products were obtained after purification by column chromatography (1–5% Either in hexane).

*4.1.1. 3-(dibromomethylene)isobenzofuran-1(3H)-one (**A**).* Yield 62%. ^{1}H NMR (DMSO-d6, 400 MHz) δ 8.36 (d, J = 8.0 Hz, 1H), 7.97 (d, J = 7.7 Hz, 1H), 7.91 (t, J = 8.0 Hz 1H), 7.75 (t, J = 8.0 Hz, 1H). ^{13}C NMR (DMSO-d6, 100 MHz) δ 164.53, 146.01, 136.32, 136.17, 131.93, 126.55, 125.86, 124.24, 77.69.

*4.2. General procedure for the preparation of (**QS-M: 1-12**)*

Under an argon atmosphere, **A** (152 mg, 0.5 mmol), $AsPh_3$(150 mg, 0.1 mmol), $Pd(dba)_2$. (14 mg, 0.025 mmol), and THF (4 ml) were added to an oven-dried Schlenk tube. $RSnBu_3$ (0.25 mmol) was added to the stirring mixture and then the mixture was heated to 66 ^oC for 24 h. The reaction mixture was cooled to room temperature and extracted with DCM (10 mL), washed brine, dried over $MgSO_4$, and filtered. The crude material was purified by flash chromatography on silica gel.

*4.2.1 (Z)-3-(bromo(phenyl)methylene)isobenzofuran-1(3H)-one (**QS-M: 1**)* Yield 72%. ^1H NMR (DMSO-d_6, 500 MHz) δ 7.94 (d, J = 8.0 Hz, 1H), 7.66 – 7.54 (m, 7H), 6.56 (d, J = 7.8 Hz, 1H). ^{13}C NMR (DMSO-d_6,125 MHz) δ 165.56, 144.65, 137.05, 136.15, 135.62, 131.27, 130.87, 130.15, 129.97, 126.11, 125.32, 122.47, 104.65. for [M+Na]+ $C_{15}H_9BrNaO_2^+$: 322.9678; found 322.9678.

*4.2.2 (Z)-3-(bromo(thiophen-2-yl)methylene)isobenzofuran-1(3H)-one (**QS-M: 2**)* Yield 65%. ^1H NMR (DMSO-d6, 500 MHz) δ 7.97 (d, J = 5.2 Hz, 2H), 7.74 – 7.62 (m, 2H), 7.50 – 7.44 (m, 1H), 7.26 (t, J = 4.3 Hz, 1H), 6.83 (d, J = 7.0 Hz, 1H).^{13}C NMR (DMSO-d_6,100 MHz) δ 165.25, 146.30, 137.14, 136.74, 135.82, 131.69, 131.59, 131.40, 128.59, 126.18, 125.38, 122.66, 96.26. for [M+Na]+ $C_{13}H_7BrNaO_2S^+$: 328.9242; found 328.9242.

*4.2.3 (Z)-3-(bromo(furan-2-yl)methylene)isobenzofuran-1(3H)-one (**QS-M:3**)* Yield 62%. ^1H NMR (DMSO-d_6, 400 MHz) δ 8.02 (s, 1H), 7.98 (d, J = 7.5 Hz, 1H), 7.75 (m, 2H), 7.34 (d, J = 7.9 Hz, 1H), 7.03 (d, J = 3.3 Hz, 1H), 6.80 – 6.74 (m, 1H). ^{13}C NMR (DMSO-d_6,125 MHz) δ 165.13, 146.74, 146.04, 136.34, 135.96, 131.88, 126.24, 125.45, 123.02, 115.35, 112.89, 93.11, 79.25. for [M+Na]+ $C_{13}H_7BrNaO_3^+$: 312.9470; found 312.9471.

*4.2.4 (Z)-3-(bromo(4-chlorophenyl)methylene)isobenzofuran-1(3H)-one (**QS-M: 4**)* Yield 59%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.96 (m, 1H), 7.71 – 7.59 (m, 6H), 6.83 – 6.50 (m, 1H). ^{13}C NMR (DMSO-d_6,125 MHz) δ 165.49, 145.00, 136.88, 135.83, 135.58, 135.04, 132.16, 131.41, 130.13, 126.17, 125.34, 122.62, 103.14. for [M+H]+ $C_{15}H_9BrClNaO_2^+$: 334.9469; found 334.9469.

*4.2.5 (Z)-3-(bromo(4-chlorophenyl)methylene)isobenzofuran-1(3H)-one (**QS-M: 5**)* Yield 75%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.97 – 7.90 (m, 1H), 7.63 (m, 4H), 7.46 – 7.38 (m, 2H), 6.64 – 6.56 (m, 1H). ^{13}C NMR (DMSO-d_6,125 MHz) δ 165.53, 163.40 (d, J = 248.6 Hz), 144.93, 136.98, 135.77, 132.73 (d, J = 8.9 Hz), 132.56, 131.33, 126.14, 125.32, 122.58, 117.15 (d, J = 22.0 Hz), 103.43.for [M+Na]+ $C_{15}H_8BrFNaO_2^+$: 340.9584; found 340.9584.

*4.2.6 (Z)-3-(bromo(4-(trifluoromethyl)phenyl)methylene)isobenzofuran-1(3H)-one (**QS-M: 6**)* Yield 67%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.99 – 7.94 (m, 3H), 7.85 (s, 1H), 7.83 (s, 1H), 7.68 – 7.62 (m, 2H), 6.66 – 6.62 (m, 1H). ^{13}C NMR (DMSO-d_6,125 MHz) δ 165.40, 145.36, 140.33, 136.74, 135.88, 131.56, 131.31, 131.04(q, J = 11.25 Hz), 126.94 (q, J = 3.75 Hz), 126.24, 125.43, 124.19 (q, J = 268.75 Hz), 122.57, 102.51. for [M+H]+ $C_{16}H_9BrF_3O_2^+$: 368.9733; found 368.9733.

*4.2.7 (Z)-3-(bromo(pyridin-2-yl)methylene)isobenzofuran-1(3H)-one (**QS-M: 7**)* Yield 48% . ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.78 (d, J = 4.5 Hz, 1H), 8.04 (t, J = 7.7 Hz, 1H), 7.96 (d, J = 7.3 Hz, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.71 – 7.55 (m, 3H), 6.65 (d, J = 7.7 Hz, 1H). ^{13}C NMR (DMSO-d_6,125 MHz) δ 165.28, 153.65, 150.67, 145.79, 138.54, 136.66, 131.61, 126.14, 126.04, 125.50, 125.43, 125.43, 122.94, 104.07. for [M+Na]+ $C_{14}H_8BrNNaO_2^+$: 323.9631; found 323.9631.

4.2.8 (E)-3-(bromo(pyridin-2-yl)methylene)isobenzofuran-1(3H)-one ***(QS-M: 8)*** Yield 32%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.72 (d, J = 7.7 Hz, 2H), 8.09 – 7.77 (m, 5H), 7.44 (m, 1H).^{13}C NMR (,125 MHz) δ 165.56, 153.10, 149.84, 144.72, 138.51, 136.45, 135.01, 131.26, 126.10, 125.99, 125.95, 125.70, 123.71, 108.37. for [M+H]+ $C_{14}H_9BrNO_2^+$: 301.9811; found 301.9811.

4.2.9 (Z)-3-(bromo(pyridin-3-yl)methylene)isobenzofuran-1(3H)-one ***(QS-M: 9)*** Yield 32% . ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.78 (m, 2H), 8.06 (d, J = 7.9 Hz, 1H), 7.98 (d, J = 6.5 Hz, 1H), 7.73 – 7.52 (m, 3H), 6.59 (d, J = 7.0 Hz, 1H). ^{13}C NMR ($CDCl_3$-d, 100 MHz) δ 165.27, 150.96, 150.26, 145.72, 137.70, 136.79, 134.69, 130.60, 126.03, 125.78, 122.19, 100.96.for [M+H]+ $C_{14}H_9BrNO_2^+$: 301.9811; found 301.9811.

*4.2.10 (E)-3-(bromo(pyridin-3-yl)methylene)isobenzofuran-1(3H)-one****(QS-M: 10)*** Yield 12.8%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.88 (s, 1H), 8.67 (d, J = 8.0 Hz, 1H), 8.61 (d, J = 3.7 Hz, 1H), 8.12 – 7.96 (m, 3H), 7.83 (t, J = 7.4 Hz, 1H), 7.55 (m, 1H). ^{13}C NMR (CDCl3-d, 125 MHz) δ 165.31, 150.52, 149.59, 143.86, 137.92, 137.17, 134.82, 131.05, 125.99, 125.60, 125.39, 104.09. for [M+H]+ $C_{14}H_9BrNO_2^+$: 301.9811; found 301.9811.

4.2.1 (Z)-3-(bromo(pyrimidin-5-yl)methylene)isobenzofuran-1(3H)-one ***(QS-M: 11)*** Yield 45%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.40 (s, 1H), 9.12 (s, 2H), 8.06 – 7.94 (m, 1H), 7.76 – 7.60 (m, 2H), 6.74 (d, J = 6.8 Hz, 1H). ^{13}C NMR (DMSO-d_6,125 MHz) δ 165.26, 159.72, 158.30, 146.62, 136.58, 136.07, 131.83, 131.38, 126.43, 125.37, 122.64, 97.16. for [M+H]+ $C_{13}H_8BrN_2O_2^+$: 302.9764; found 302.9764.

4.2.12 (E)-3-(bromo(pyrimidin-5-yl)methylene)isobenzofuran-1(3H)-one ***(QS-M: 12)*** Yield 7.6%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.21 (s, 1H), 9.13 (s, 2H), 8.68

(d, J = 8.0 Hz, 1H), 8.12 – 7.98 (m, 2H), 7.86 (t, J = 7.5 Hz, 1H). ^{13}C NMR ($CDCl_3$-d,125 MHz) δ 158.15, 158.11, 157.29, 137.56, 135.03, 131.54, 131.18 – 130.93, 126.20, 125.51, 125.49, 100.05. for [M+H]+ $C_{13}H_8BrN_2O_2^+$: 302.9764; found 302.9764.

*4.2. procedure for the preparation of (**QS-M: 13**)*

Under an argon atmosphere, **A** (152 mg, 0.5 mmol), $HSnBu_3$(0.07 ml, 0.25 mmol), $Pd(PPh_3)_4$. (58 mg, 0.05 mmol), and THF (4 ml) were added to an oven-dried Schlenk tube. $HSnBu_3$ (0.07 ml, 0.25 mmol) was added to the stirring mixture and then the mixture was heated to 66 ^oC for 24 h. The reaction mixture was cooled to room temperature and extracted with DCM (10 mL), washed brine, dried over $MgSO_4$, and filtered. The crude material was purified by flash chromatography on silica gel.

*4.2.1 (Z)-3-(bromomethylene)isobenzofuran-1(3H)-one (**QS-M: 13**)* Yield 25%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.02 (d, J = 7.8 Hz, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.87 (t, J = 2.5 Hz,1H), 7.69 (t, J = 7.5 Hz, 1H).^{13}C NMR ($CDCl_3$-d,125 MHz) δ 165.24, 148.90, 137.94, 134.81, 130.53, 125.86, 124.45, 119.92, 85.63. for [M+H]+ $C_9H_6BrO_2^+$:224.9546; found 224.9546.

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국문초록

새로운 효과적인 쿼럼센싱 저해제로서의

브롬화 이소벤조퓨라논 합성

일련의 브롬화 이소벤조퓨라논 유도체를 설계하고 합성하였다. 이 화합물은 생물막 억제 활성을 가지며 쿼럼센싱 저해제 (QSI)의 좋은 후보 물질이다. 이들 모두는 두 단계로 간단히 합성할 수 있다. 첫 번째 단계로 최적인 phthalic anhydride의 디브로모아올레핀화반응은 PPh_3를 $P(O\textit{i}\text{-}Pr)_3$으로 대체하고 Zn을 첨가하여 진행되었고, 반응 수율을 크게 증가시켰다. 그 다음에 아릴틴 화합물과 디브로민화 퓨라논 (A)의 Stille 커플링 후, 주생성물로 Z형 이성질체를 합성하였다. Z 이성질체의 형성은 ^1H NMR 분광법과 단결정 X-선 회절을 통해 확인하였다. 일련의 아릴치환 브로모퓨라논을 제조하고 쿼럼센싱 억제에 대해 시험하였다. 그 실험된 화합물들 중 페닐치환된 화합물이 가장 활성이 있는 것으로 판명되었다.

주요어 : 쿼럼센싱 저해제, 치주염, 박테리아 생물막, 브롬화 퓨라논